Macrophage responses under hypoxic conditions – a drug target for the discovery of anti-cancer agents?

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Introduction

• Within tumors there are localized areas of low oxygen (hypoxia) to which monocytes are recruited and differentiate into tumor associated macrophages (TAM’s)
• Under hypoxic conditions macrophages take on a pro-tumor phenotype, secreting cytokines and creating a micro-environment to promote angiogenesis and immune evasion of tumor cells
• Targeting TAM’s to prevent or reverse their anti-inflammatory pro-tumor phenotype represents a potential anti-cancer therapy
• The aim of this study is to develop a physiologically relevant human cell-based assay to screen a panel of small molecules for anti-tumor effects

Methods

• Macrophage differentiation - 1x10^7 THP-1/U937 human monocytic cells were cultured in RPMI + 10% FBS +/-200ng/ml Phorbol 12-myristate 13-acetate (PMA) for 3 days, washed and rested for a further 5 days. Cells were characterised by flow cytometry (FC).
• Macrophage treatment – 1x10^6 THP-1/U937 cells were treated with 200nM PMA to generate a differentiated macrophage phenotype. Polarised macrophages were treated for 72h (see below), characterised by FC and soluble factors in the supernatant measured by AlphaLISA (PerkinElmer) or ELISA (R&D Systems).

Treatment
• Normoxia - 20% Oxygen
• Hypoxia - 1% Oxygen
• Lactate - 5µM, 50µM, 500µM
• M2 - IL-4 (100ng/mL)+IL-13 (100ng/mL)
• M1 - LPS (100ng/mL)+ IFNγ (20ng/mL)

Cancer cell line response to hypoxia – 1x10^6 A172/A549 human cancer cell lines were treated with 1% oxygen (hypoxia) for 48h. Glucose and lactate measured in the media using luminescent assay kits (Promega).

Results 1 – Macrophage differentiation

THP-1 and U937 cells were differentiated into a macrophage phenotype as determined by increased adherence and granularity in culture. Macrophages up-regulated expression of surface CD14, CD11b, TLR2, CD206 and intracellular lysosomes.

Results 2 – Macrophage response to hypoxia and lactate

Lactate treated cells express low levels of pro-inflammatory markers/do not demonstrate an M2 phenotype.

Results 3 – Cancer cell line response to hypoxia

In hypoxia, cancer cell lines altered their glycolytic pathway as demonstrated by increased glucose uptake and lactate secretion.

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Summary and Conclusion

• THP-1 and U937 cells were differentiated into a macrophage phenotype. When exposed to lactate but not hypoxia, U937 macrophages increased expression of VEGF. Lactate treated macrophages displayed low levels of pro-inflammatory markers compared to M1 polarized macrophages, consistent with a pro-tumor phenotype that could promote angiogenesis and immune evasion of tumor cells.

• A172 and A549 cancer cell lines altered their glycolytic pathway and secreted increased lactate in response to hypoxia. Together the data support the notion that macrophages respond to hypoxia indirectly through the lactate secreted by cancer cells. This is an important consideration when developing physiologically relevant human cell-based assay to screen small molecules for anti-tumor effects on macrophages.